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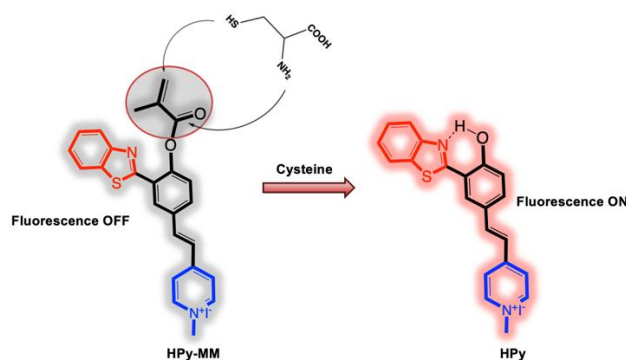
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Design, Synthesis, and Cytotoxicity Assessment of a Novel Fluorescent Probe for Cysteine Detection

Mehmet Oguz
Selcuk University

Abstract: Cysteine (Cys) is a sulfur-containing amino acid that plays a vital role in numerous biological processes, including protein synthesis, redox homeostasis, and cellular metabolism. Abnormal Cys levels have been associated with various diseases, creating a growing demand for the development of fluorescent probes for its detection. In this study, a new pyridinium–benzothiazole-based fluorescent probe was designed and synthesized as a potential molecular platform for Cys sensing. The structure of the synthesized compound was successfully confirmed by ^1H and ^{13}C NMR spectroscopy. To evaluate its suitability for biological applications, cytotoxicity studies were carried out, and the probe exhibited favorable biocompatibility under the tested conditions. These results demonstrate that the synthesized probe is a suitable candidate for further investigations in fluorescence-based sensing studies. Overall, the newly developed pyridinium–benzothiazole derivative provides a useful foundation for the future development of Cys-responsive fluorescent probes and related biological applications.

Keywords: Cysteine, Fluorescent probe, Cytotoxicity



Introduction

Cysteine (Cys) is a biologically important sulfur-containing amino acid that participates in a wide range of physiological processes (Fang et al., 2020; Jung et al., 2012). As a precursor of glutathione, it plays a central role in maintaining intracellular redox balance and protecting cells against oxidative stress. In addition, Cys contributes to protein biosynthesis, enzymatic regulation, metal ion coordination, and cellular signaling pathways through its highly reactive thiol group (Tang et al., 2026; Kim et al., 2026). Since the intracellular concentration of Cys is tightly regulated, its imbalance has been associated with the onset and progression of several pathological conditions, including neurodegenerative disorders, liver dysfunction, cardiovascular diseases, and cancer (Gao et al., 2011; Mahmoud et al., 2016). Therefore, the reliable detection of Cys has attracted considerable interest in biomedical research, clinical diagnostics, and analytical chemistry.

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A variety of analytical techniques have been developed for the determination of Cys, including high-performance liquid chromatography (HPLC), capillary electrophoresis, electrochemical methods, and mass spectrometry (Lalitha et al., 2026; Asghar et al., 2026). Although these techniques provide reliable analytical performance, they generally require sophisticated instrumentation, time-consuming sample preparation, or trained personnel, which may limit their use in routine and real-time analyses. Fluorescence-based methods have emerged as an attractive alternative because of their high sensitivity, rapid response, operational simplicity, and capability for non-invasive detection (Jiang et al., 2025; Luo et al., 2024). Furthermore, fluorescent probes enable real-time monitoring of biological analytes with high spatial and temporal resolution, making them valuable tools for biochemical research and the development of analytical sensing platforms.

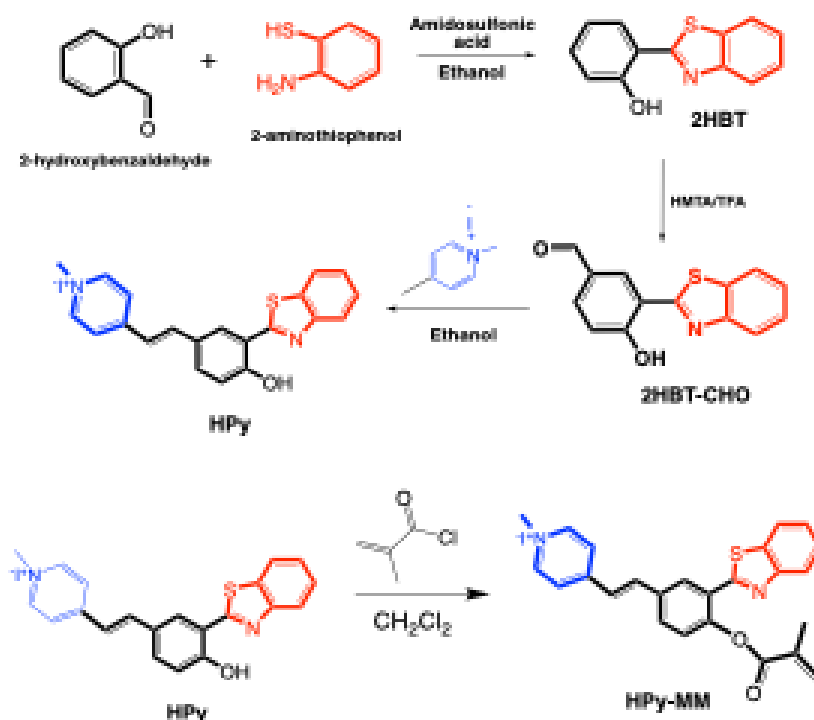
Among the various fluorophore scaffolds currently available, benzothiazole derivatives have attracted considerable attention owing to their favorable photophysical properties, synthetic accessibility, and structural versatility. Their conjugated framework enables efficient fluorescence emission, while the molecular structure can be readily modified to tailor the optical response toward specific analytes. Likewise, pyridinium derivatives are frequently incorporated into fluorescent probe design because of their strong electron-withdrawing character, good water compatibility, and ability to facilitate intramolecular charge transfer (ICT) processes (Lee et al., 2026; Erdemir et al., 2026; Kaur et al., 2024). The combination of benzothiazole and pyridinium moieties offers a rational molecular framework for the design of fluorescent probes with desirable optical characteristics and potential applicability in biological sensing.

In the present study, a new pyridinium–benzothiazole-based fluorescent probe was designed and synthesized as a potential platform for Cys sensing. The synthesized compound was structurally characterized by ^1H and ^{13}C NMR spectroscopy to confirm its molecular structure. Furthermore, cytotoxicity studies were performed using the Alamar Blue assay to evaluate its biocompatibility and assess its suitability for potential biological applications. The results of this work provide a useful foundation for the future development of Cys-responsive fluorescent probes and related biological studies.

Experimental Section

Materials and Methods

The intermediates 2HBT, 2HBT-CHO, and HPy were synthesized according to previously reported methods with slight modifications (Erdemir et al., 2022; Sahana et al., 2015). These intermediates were subsequently used for the synthesis of the target pyridinium–benzothiazole fluorescent probe (Scheme 1).



Scheme 1. Synthetic pathway for the preparation of HPy-MM

Synthesis of Hpy-MM

Hpy (1.0 mmol) and methyl methacrylate (1.1 mmol) were dissolved in dichloromethane (20 mL), and three drops of triethylamine were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 h. After extraction with water, the product was purified by column chromatography (CH_2Cl_2) to afford Hpy-MM in 65% yield. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.94 (d, 2H, $J=6.6$ Hz), 8.25–8.31 (m, 3H), 8.15–8.19 (m, 2H), 8.01 (d, 1H, $J=7.8$ Hz), 7.85 (d, 1H, $J=16.5$ Hz), 7.63–7.67 (m, 2H), 7.58 (t, 1H, $J=7.2$ Hz), 7.50 (t, 1H, $J=7.2$ Hz), 6.48 (s, 1H), 6.06 (s, 1H), 4.30 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 165.7, 162.3, 152.9, 152.0, 146.9, 146.0, 135.4, 135.1, 133.0, 132.2, 130.8, 130.7, 129.8, 127.8, 127.3, 126.5, 124.6, 123.4, 122.7, 47.6, 18.7.

Cell Viability Assay

Cytotoxicity Assay. The biocompatibility of Hpy-MM was assessed in PNT1A and HeLa cells using the Alamar Blue assay. Briefly, cells were seeded into 96-well plates at a density of 1×10^4 cells per well and allowed to attach for 24 h at 37 °C. The cells were subsequently exposed to various concentrations of Hpy-MM (0–200 μM) for 48 h. Following the treatment period, Alamar Blue reagent was added at 10% of the culture medium volume, and the plates were incubated for an additional 4 h. Absorbance was recorded at 570 and 600 nm using a microplate reader. Cell viability was expressed relative to untreated control cells, and IC_{50} values were determined from dose–response curves generated from three independent experiments.

Results and Discussion

Synthesis and Structural Characterization of Hpy-MM

Hpy-MM was synthesized through a multistep synthetic route involving pyridinium and benzothiazole intermediates, as illustrated in Scheme 1. The final product was isolated by column chromatography and structurally characterized by ^1H and ^{13}C NMR spectroscopy (Figs. 1–2). The NMR data were consistent with the proposed molecular structure of Hpy-MM. In the ^1H NMR spectrum, the methyl protons of the pyridinium moiety appeared at δ 4.23 ppm, while the methyl protons of the methacryloyl group were observed at δ 2.15 ppm. In addition, the characteristic aromatic proton signals corresponding to the pyridinium unit were detected in the expected chemical shift region. Further structural confirmation was provided by the ^{13}C NMR spectrum, in which the carbonyl ($\text{C}=\text{O}$) carbon of the methacryloyl group was observed at δ 167.24 ppm, supporting the successful incorporation of the methacryloyl functionality into the target molecule.

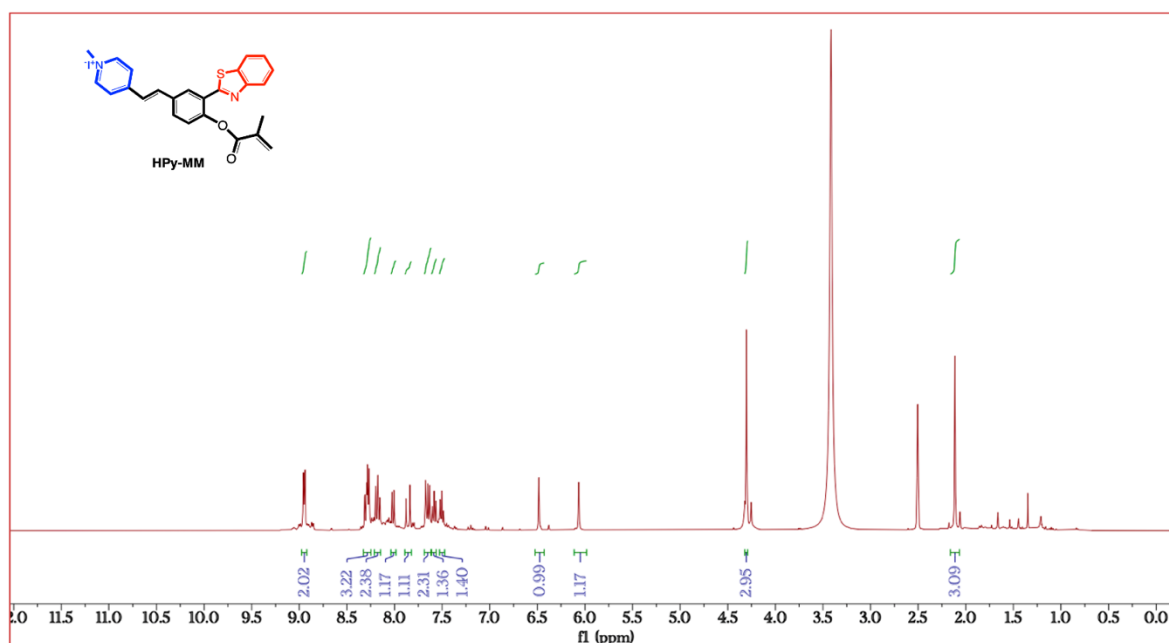
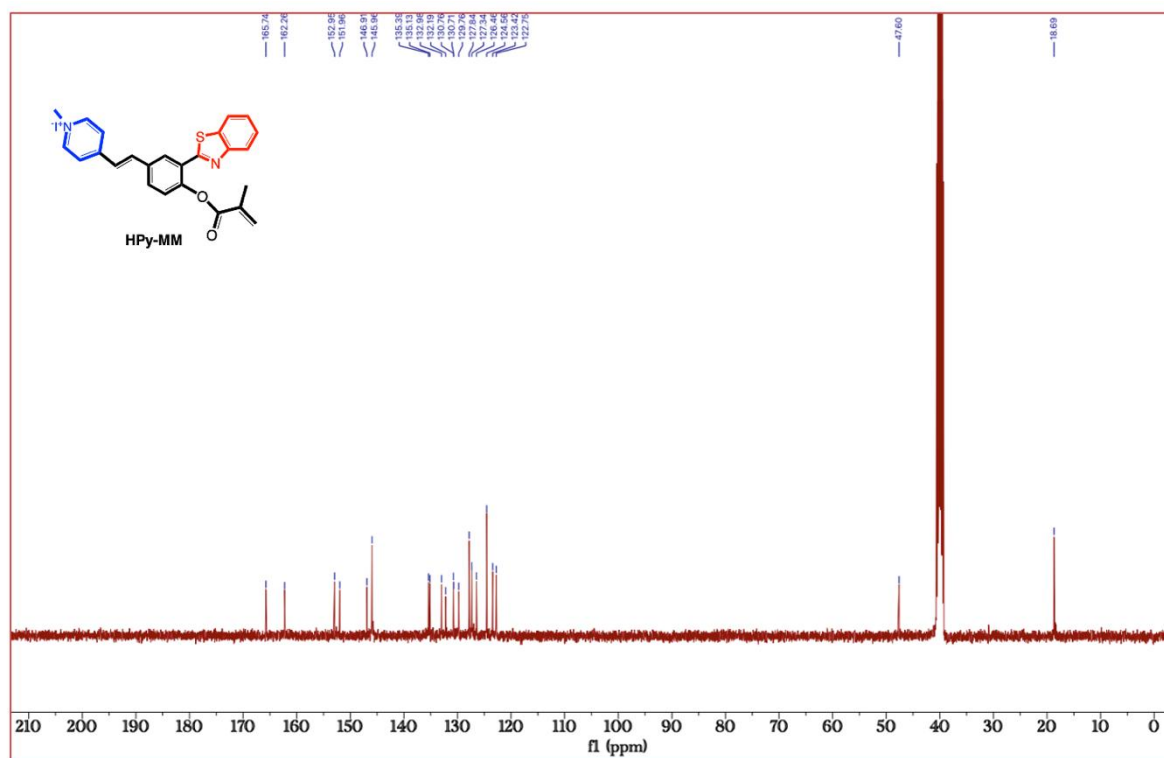
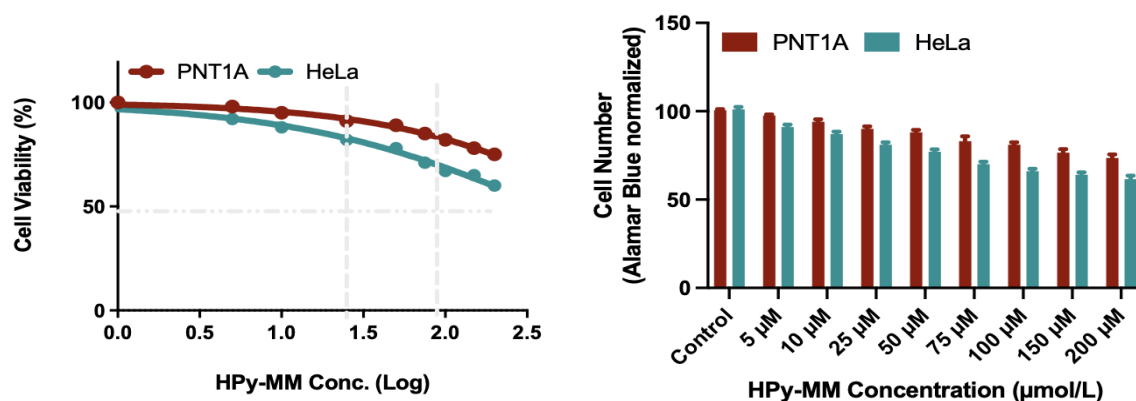


Figure 1. ^1H NMR spectrum of Hpy-MM ($\text{DMSO}-d_6$, 400 MHz)

Figure 2. ^{13}C NMR spectrum of **HPy-MM** (DMSO- d_6 , 100 MHz)

Cytotoxicity Evaluation

Figure 3. IC_{50} values of the **HPy-MM** on PNT1A and HeLa

The cytotoxicity of HPy-MM was evaluated in PNT1A and HeLa cells using the Alamar Blue assay, and the results are presented in Fig. X. As the concentration of HPy-MM increased, a gradual reduction in cell viability was observed in both cell lines. Nevertheless, cell viability remained above approximately 85% in PNT1A cells and above 75% in HeLa cells, even at the highest tested concentration (200 μM), indicating that HPy-MM exerts only limited cytotoxic effects under the investigated conditions. The calculated IC_{50} values further supported these observations, with values exceeding 200 μM for PNT1A cells and 195.1 μM for HeLa cells (Table 1). These findings indicate that HPy-MM possesses good biocompatibility and may be considered suitable for further biological studies.

Table 1. Calculated IC_{50} values (μM) for **HPy-MM**.

Compound	PNT1A	HeLa
HPy-MM	> 200	195.1

Conclusion

In this study, a new pyridinium–benzothiazole-based fluorescent compound, HPy-MM, was successfully synthesized through a multistep synthetic route. The molecular structure of the synthesized compound was confirmed by ^1H and ^{13}C NMR spectroscopy, verifying the successful formation of the target molecule. To evaluate its potential for biological applications, the cytotoxicity of HPy-MM was investigated using the Alamar Blue assay in PNT1A and HeLa cell lines. The results demonstrated that the compound maintained high cell viability over the investigated concentration range, indicating favorable biocompatibility. These findings suggest that HPy-MM represents a suitable molecular platform for future studies involving cysteine-responsive fluorescent probe development and related biological applications.

Scientific Ethics Declaration

* The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

Conflict of Interest

* The author declares that there is no conflict of interest

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Author(s) Information

Mehmet Oguz

Selcuk University, Faculty of Science
Department of Chemistry, Konya/Türkiye
ORCID iD: <https://orcid.org/0000-0002-3999-620X>
Contact e-mail: commoguz@selcuk.edu.tr

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